

LAW OFFICES
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC

2100 PENNSYLVANIA AVENUE, N.W.
WASHINGTON, D.C. 20037-3202
TELEPHONE (202) 293-7060
FACSIMILE (202) 293-7860

CALIFORNIA OFFICE

1010 EL CAMINO REAL
MENLO PARK, CA 94025
TELEPHONE (650) 325-5800
FACSIMILE (650) 325-6606

ATTN: BOX PCT

Assistant Commissioner for Patents
Washington, D.C. 20231

September 7, 1999

JAPAN OFFICE

TOEI NISHI SHIMBASHI BLDG. 4F
13-5 NISHI SHIMBASHI 1-CHOME
MINATO-KU, TOKYO 105, JAPAN
TELEPHONE (03) 3503-3760
FACSIMILE (03) 3503-3756

ATTN: BOX PCT

Re: Application of Susumu IKEHARA, Kikuya SUGIURA, Tienan JIN, Haruo MORITA, Shinji SOGO,
Kazuya YAMANISHI and Masakazu ADACHI
IMMUNOTOLERANCE INDUCER
Our Reference: Q55691
PCT/JP98/00909, filed March 4, 1998

Dear Sir:

Applicants herewith submit the attached papers for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty. Attached hereto is the application identified above which is a verified translation of PCT International Application No. PCT/JP98/00909, filed March 4, 1998, comprising the specification, claims, two (2) sheets of drawings, executed Declaration and Power of Attorney, International Search Report, executed Assignment and PTO Form 1595.

The Government filing fee is calculated as follows:

Total Claims	9 - 20 =	0 x \$18 =	\$ 000.00
Independent Claims	4 - 3 =	1 x \$78 =	\$ 78.00
Base Filing Fee	(\$840.00)		\$ 840.00
Multiple Dep. Claim Fee	(\$260.00)		\$ 260.00
TOTAL FILING FEE			\$ 1178.00
Recordation of Assignment Fee			\$ 40.00
TOTAL U.S. GOVERNMENT FEE			\$ 1218.00

Checks for the statutory filing fee of \$ 1,178.00 and Assignment recordation fee of \$ 40.00 are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.16 and 1.17 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

Japanese Patent Application
52930/1997
155015/1997
346737/1997

Filing Date
March 7, 1997
June 12, 1997
December 16, 1997

The Office is invited to contact the above firm on any question which might arise on the above-named application. Any contact that the Office might need to make should be directed to the undersigned at (202)293-7060.

Respectfully submitted,
SUGHRUE, MION, ZINN, MACPEAK & SEAS
Attorneys for Applicant(s)

By

Waddell A. Biggart

Registration No. 24,861

WAB:clf

IMMUNOTOLERANCE INDUCER

TECHNICAL FIELD

This invention relates to an immunotolerance inducer capable of organ transplantation and more particularly to an immunotolerance inducer capable of inducing the immunological tolerance necessary for the maintenance of transplanted organs.

BACKGROUND ART

The immunosuppressant is an indispensable adjunct to organ transplantation and novel immunosuppressants are being developed one after another. According to the intended application (use), immunosuppressants can be classified into two categories. Drugs of one category are those which are taken daily as long as the graft remains in the recipient's body for the prophylactic suppression of graft rejection and are variously called maintenance immunosuppressants, prophylactic immunosuppressants, and basal immunosuppressants. Drugs of the other category are those used in massive doses, though for limited periods of time, with an aim to causing an intensive immunosuppression necessary for the cure of the rejection response which may occur notwithstanding sustained immunosuppression, chiefly cellular rejection, and are known as therapeutic drugs for graft rejection.

09380579 090799

However, in terms of the principal pharmacologic action as well as side effects, those immunosuppressants can hardly be considered harmless to the human body, and since long-term maintenance doses and/or high doses are required, toxic effects and/or adverse drug reactions which cannot be disregarded are inevitable. Furthermore, those immunosuppressants, when used independently, are not potent enough to produce sufficient immunosuppression, nor are they capable of curing graft rejections after onset at a high cure rate.

Meanwhile, there are clinical reports, though sporadically, on the success with which grafts were maintained even without administration of an immunosuppressant, and those favorable outcomes have been attributed to the induction of immunological tolerance. If such immunotolerance could be actually established, administration of immunosuppressants would not be necessary. Therefore, the artificial induction of immunological tolerance is regarded as a supreme objective in organ transplantation today and results of many relevant studies have been reported.

On the methodology for artificial induction of immunological tolerance, reference may be made to the following report, among others.

Induction of Tolerance by Transfer of a Splenocyte

or Myelocyte Tolerogen in Combination with Administration of an Antimitotic Drug [Fukuoka Acta Med., 81(1), 20-40 (1990); Microbiol. Immunol., 32(3), 283-292 (1988), etc.].

As the antimitotic drug, 6-mercaptopurine, methotrexate, cyclophosphamide (CP), 5-fluorouracil, azathioprine (AZP) and procarbazine are mentioned, and it is warned that cyclosporin A (CsA) and steroids, which are remote from those antimitotic agents in the mode of action, are not suitable for the induction of immunological tolerance.

Hayakawa et al. reported their attempt to induce a donor-specific immunocompromised state with FK506 [Keio Medicine, 72(3), 163-176 (1995)]. Similarly Muramatsu et al. reported on the possibility of inducing immunological tolerance with 15-DSG [Abstract of Papers read before the 20th Congress of the Japan Society of Microsurgery, 89-90 (1994)].

The present inventors reported previously that, in mice, administration of bone marrow cells (particularly hematopoietic stem cells) into the portal vein or by the usual intravenous route results in entrapment of the donor-derived cells in the recipient's liver, establishment of chimerism and induction of immunotolerance [Eur. J. Immunol., 24, 1558 (1994)].

0930579 090799
66/060" 6/503660

The object of this invention is to provide a technology by which the necessary immunological tolerance for organ transplantation can be successfully established. Stated differently, this invention has for its object to provide a novel method for ensuring a positive sustenance of grafts without use of immunosuppressants for maintenance (by long-term administration of an immunosuppressant) and, hence, without risks for serious side effects.

After an intensive study the inventors found that the pharmaceutical regimen described hereinafter meets the above object and have perfected this instant invention.

DISCLOSURE OF THE INVENTION

The invention provides an immunotolerance inducer for inducing immunological tolerance in a patient undergoing an organ transplantation comprising a first pharmaceutical composition for portal administration which comprises an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells, mature lymphocytes or a mixture thereof in combination with a pharmaceutical carrier and a second pharmaceutical composition for intravenous administration which comprises an effective amount of said tolerogen in combination with a pharmaceutical carrier, and more particularly to said immunotolerance inducer

66/060" 6/508660

comprising a bone marrow cell fraction as said tolerogen.

Furthermore, the present invention provides an immunotolerance inducer for application to a patient undergoing an organ transplantation in association with radiation for inducing immunological tolerance in said patient comprising an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells or a mixture thereof in combination with a pharmaceutical carrier, more particularly said immunotolerance inducer comprising a bone marrow cell fraction as said tolerogen.

By using the immunotolerance inducer of this invention, the immunological tolerance meeting the above-mentioned object can be successfully established so that the transplanted organ can be maintained in satisfactory condition.

As described above, the first-mentioned immunotolerance inducer of this invention essentially comprises said first pharmaceutical composition for portal administration and said second pharmaceutical composition for intravenous administration and as far as this constitution is retained, the pharmaceutical artefact and dosage form of each composition are not particularly restricted.

For example, said first and second pharmaceutical

09380579-090799
662060-62508860

compositions may be provided in a single dosage form or optionally in independent dosage forms. Thus, as typically represented by the examples of use given hereinafter for the immunotolerance inducer of this invention, there is no particular limitation on the type of pharmaceutical artefact and dosage form only provided that the object of inducing the necessary immunological tolerance is accomplished.

The tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells, mature lymphocytes or a mixture thereof, which is the active ingredient in said first and second pharmaceutical compositions in common may for example be a tolerogen derived from the graft donor (an animal of the same strain as the donor). The active ingredient mentioned above may be a bone marrow cell fraction, spleen cell fraction, peripheral blood cell fraction or a fraction comprising a mixture of them, which contains said cells.

The separation and isolation of such tolerogens can be carried out by known procedures. For example, the procedure described by Yamamoto et al. [Blood, 88, 445-454 (1996) and the procedure described in Protocols in Experimental Cellular Immunity [ed. by Mishell B. B., Shiigi S. M.; translated by Katsuyuki Imai, Susumu Kawaguchi and Takayuki Harada, Rikogaku-Sha, pp.3-12,

09380579 090799
552060 52508660

1982] can be employed.

The preferred tolerogen from the graft donor (a human) includes bone marrow cells and peripheral blood cells. The method of harvesting those cells is well known to those skilled in the art. For example, the method for handling bone marrow cells may be the same as that used in bone marrow transplantation.

The tolerogen for use in the first pharmaceutical composition is preferably a bone marrow cell fraction rich in hematopoietic progenitor cells, a spleen cell or peripheral blood cell fraction containing mature lymphocytes (exclusive of activated lymphocytes) or a mixture thereof. On the other hand, the tolerogen for the second pharmaceutical composition is preferably said bone marrow cell fraction. As the active component of said first and second pharmaceutical compositions, a bone marrow cell fraction is preferred as mentioned above but the peripheral blood cell fraction containing the hematopoietic stem cells mobilized from the bone marrow by cytokines such as G-CSF is also preferred partly because it contains both of mature lymphocytes and hematopoietic progenitor cells and partly because such a cell fraction is readily available.

To provide each of said first and second pharmaceutical compositions, the active component can be

09380579 090799
662060 62508860

formulated into a conventional dosage form known for pharmaceutical products containing cellular fractions of this type. The dosage form can be judiciously selected from among a variety of dosage forms for the present purpose. An injectable dosage form can be mentioned as an example. The pharmaceutical carrier or vehicle which can be used in the manufacture of such dosage forms includes a broad range of pharmaceutically acceptable substances. The method of preparation may also follow the established pharmaceutical procedures. In preparing such dosage forms, the various infusions which are in broad use nowadays can also be employed.

In the practice of this invention, said dosage forms can be prepared extemporaneously, if desired, on the occasion of organ transplantation, using the material obtained from the graft donor.

For administration of the first and second pharmaceutical compositions of this invention, it is essential that the first pharmaceutical composition be administered into the portal vein and the second pharmaceutical composition intravenously. The dosage and timing of administration of each pharmaceutical composition are not particularly restricted but can be judiciously elected by those skilled in the art only provided that the necessary immunological tolerance may

be successfully established.

A representative treatment modality comprises administering the first pharmaceutical composition into the portal vein and thereafter administering the second pharmaceutical composition intravenously. The intravenous administration of the second pharmaceutical composition is preferably carried out at the time when, in the mixed lymphocyte reaction of spleen cells, the reactivity of the host's cells to the donor's alloantigen has decreased to a minimum once (e.g. around the 4th day in mice) and then begins to increase again (around the 5th day in mice).

The recommended dosage of the first pharmaceutical composition which is administered into the portal vein is the minimum dose (3×10^7 cells in mice) required to ensure that said reactivity to the donor's alloantigen in the mixed lymphocyte reaction after portal administration becomes minimal (maximum inhibition of the reaction) and plateau out.

The recommended dosage of the second pharmaceutical composition which is administered intravenously is approximately the dose (3×10^7 cells in mice) required for reconstructing the host's immune system in the transplantation of the ordinary major histocompatibility complex (MHC)-incompatible bone marrow (after irradiation

00330579 090799
65/060 6/503560

in a lethal dose in mice).

The mixed lymphocyte reaction test for spleen cells, referred to above, can be carried out in the routine manner [Protocols in Experimental Cellular Immunity, 147-149, 1982]. The total dosage for portal and intravenous administration in humans may be the dose used in the conventional bone marrow transplantation. For example, the dosage in terms of bone marrow cells may be about 3×10^8 cells/kg or more.

By the portal administration of said first pharmaceutical composition and subsequent intravenous administration of said second pharmaceutical composition according to this invention, the desired immunological tolerance can be induced to ensure a satisfactory maintenance of the transplanted organ.

This invention, therefore, provides a method of inducing immunological tolerance using the specific procedures described above.

The above-mentioned result achieved by the method of this invention is not related to the timing of transplantation of a graft material. Thus, the transplantation procedure can be successfully carried out, whether in parallel with the procedure of this invention or after the establishment of immunological tolerance by the procedure of this invention.

55/050" 52503E50

In inducing immunological tolerance in accordance with this invention, various other medical treatments and administration of drugs, which are concomitantly practiced in procedures of this kind can be practiced in combination with the procedure of the invention unless the effect of the invention is thereby compromised.

As an example, administration of said immunosuppressants can be mentioned. The method, dosage, and timing of administration of immunosuppressants can be judiciously selected by those skilled in the art.

The particularly preferred immunosuppressant includes cyclosporin A and FK506, to mention just a few representative drugs, and the dosage and administration method may be those recommended for the known commercial products. The particularly preferred mode of administration is to administer an immunosuppressant shortly after administration of the first pharmaceutical composition, once or twice, for example around the 2nd day or around the 2nd and 5th days following portal administration.

Furthermore, the present invention provides an immunotolerance inducing technology capable of introducing chimerism with a minimum of invasion and with a high degree of certainty to ensure a long-term sustenance of the state of immunological tolerance.

Thus, the present invention provides an immunotolerance inducer to be applied, in association with radiation, to a patient undergoing an organ transplantation for inducing immunological tolerance in the patient, which comprises an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells or a mixture thereof and a pharmaceutically acceptable carrier.

Even when the portal or intravenous administration of the immunotolerance-inducer of this invention is a single-dose administration, the immunotolerance meeting the object mentioned hereinbefore can be established as far as the procedure is followed in combination with radiation, with the result that the transplanted organ can be maintained in satisfactory condition.

The tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells or a mixture thereof which constitutes the active component of the medicine of this invention for administration to the patient in association with radiation, may for example be a bone marrow cell fraction, a peripheral blood cell fraction, or a mixture thereof, which contains hematopoietic stem cells and hematopoietic progenitor cells.

The tolerogen derived from the graft donor (a human) includes a bone marrow cell fraction, an umbilical blood

cell fraction and a peripheral blood cell fraction containing hematopoietic stem cells mobilized by a cytokine such as G-CSF.

The technique for separation and isolation of said tolerogen for use in the immunotolerance inducer of this invention to be applied in association with said radiation, the method of manufacture of the inducer, its dosage form, the pharmaceutical carrier for use in the manufacture of the dosage form, the route of administration and dosage may all be the same as those mentioned hereinbefore for the first pharmaceutical composition for portal administration and the second pharmaceutical composition for intravenous administration.

It is essential, however, that the above immunotolerance inducer of this invention be used in association with radiation, that is to say it be administered into the portal vein or intravenously to the patient given said radiation.

The reference dosage for intravenous administration is roughly the dose (3×10^7 cells in mice) required for reconstructing the host's immune system in the transplantation of the ordinary major histocompatibility complex (MHC)-incompatible bone marrow (after irradiation in a lethal dose in mice).

With the above dosage for mice being taken as a

09380579.090799

reference, the dosage of the medicine of this invention can be judiciously selected according to the conventions of bone marrow transplantation. As a specific example, the dose of about 3×10^8 cells/kg or more, in terms of bone marrow cells, can be mentioned.

The radiation mentioned above can be carried out in the conventional manner. More particularly, the patient (recipient) undergoing an organ transplantation is exposed to a suitable radiation dose, for example an at least 6.5 Gy and yet sublethal dose, preferably about 7.0 Gy, per exposure, on a total body irradiation (TBI) basis. This radiation dose is characterized also as a radiation dose providing for recovery of the recipient's bone marrow cells.

The above radiation can be carried out before administration of the medicine of this invention and usually the medicine is preferably administered within 24 hours of irradiation.

This invention is advantageous in that the expected efficacy can be obtained by a single dose of medication which is least invasive to the recipient.

By administering the medicine of this invention in conjunction with radiation, the desired immunological tolerance can be induced for a satisfactory maintenance of the transplanted organ.

The present invention, therefore, provides an immunotolerance inducing method involving radiation.

The phenomenon that the desired immunological tolerance is induced by this method involving radiation for a successful maintenance of the graft is also unrelated to the timing of the operation for transplantation of the graft.

In practicing the above combination treatment method, too, the various medical treatments and medications which are usually given in procedures of this kind, for example administration of immunosuppressive drugs such as cyclosporin A, FK506, etc., can be carried out concomitantly unless the effect of the invention is diminished or cancelled.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagrammatic representation of the engraftment rate of skin grafts in Test Example 3 and

Fig. 2 is a diagrammatic representation of the engraftment rate of skin grafts in Test Example 4.

BEST MODE FOR CARRYING OUT THE INVENTION

The following is a description of the tests performed with the active component of this invention for illustrating this invention in further detail.

Test Example 1

The induction of immunological tolerance in the test

examples was effected by (1) the injection of an allogeneic donor's spleen cells or bone marrow cells into the portal vein and (2) the intravenous injection of the allogeneic donor's bone marrow cells and the establishment of immunological tolerance was evaluated using the engraftment rate of skin grafts (allogeneic to the donor) which is the organ most susceptible to rejection as an indicator.

(1) Preparation of a spleen cell suspension

Spleen cells were harvested from 8-week-old female BALB/cCrSlc mice (body weights 19-22 g, BALB/c; Japan SLC Inc.) and loosened up with a pair of non-toothed forceps on a 200 G stainless steel mesh screen in RPMI1640 solution (Nikken Bio Med. Lab.) to prepare discrete spleen cells. The cells were washed with RPMI1640 solution once and subjected to hemolysis with Tris-HCl-ammonium chloride buffer (0.75% NH_4Cl , 0.017 M Tris-HCl, pH 7.5). After two further washings with RPMI1640, the spleen cells were resuspended in the same solution to provide a spleen cell suspension (concentration: $1.0 \times 10^8/\text{ml}$).

(2) Preparation of a bone marrow cell suspension

The femurs and tibias were isolated from 8-week-old female BALB/c mice and a 22-G needle (Code No. NN-2225R, Terumo Co., Ltd.) attached to a syringe (2.5 ml, Code No. SS-02S, Terumo Co., Ltd.) was inserted into each bone from

65/060" 6/508860

the knee joint side and the bone marrow cells were flushed into a sterilized dish (90x16 mm, Iwaki Clinical Test Ware) using RPMI1640 solution and suspended in RPMI1640 solution. The bone marrow cells thus harvested were washed with RPMI1640 solution once and resuspended in the same solution to provide the objective bone marrow cell suspension (concentration: 1.0×10^8 /ml).

(3) Injection into the portal vein

Under pentobarbital anesthesia (Pitman-Moor Inc.; 37.5 mg/kg body weight, i.p.), 10-week-old female C57BL/6CrSlc mice (B6; body weights 20-24 g, Japan SLC Inc.) were shaved of hairs with a razor and disinfected. Then, a midline incision was made in the abdominal region and the mesenterium was exposed. A 27 G needle (Terumo Co., Ltd.) attached to a 1 ml-tuberculin syringe was inserted through the adipose tissue of the mesenterium and 3×10^7 BALB/c mouse spleen cells or bone marrow cells (0.3 ml suspension) prepared in (1) above were administered into the portal vein.

(4) Intravenous injection

The bone marrow cell suspension prepared in (1) above was adjusted to a concentration of 1×10^8 cells/ml and the 3×10^7 cells equivalent of the suspension (0.3 ml) was administered into the tail vein of the host mouse at day 5 after the portal injection described in (3) above.

(5) Skin grafting

Skin grafting was carried out at day 7 after portal injection. The preparation of skin graft materials and the transplantation thereof were carried out as follows, with reference to the procedure described in the literature [Mayumi et al., Jpn. J. Surg., 18, 548-557 (1988)].

Thus, as the donor, 8-week-old BALB/c mice were sacrificed under ethyl ether (Nacalai Tesque Inc.) anesthesia. Using a depilatory cream (Feather Hair Remover, Feather Softy Razor Co., Ltd.), the whole hair coat was removed and after disinfection with 70% alcohol, the full-thickness skin layer was peeled off and recovered. After the subcutaneous adipose tissue was removed with a pair of forceps (bent tip, tapered, non-toothed) and sanitary cotton balls as much as possible, the skin was cut into a flap (1.2x1.5 cm²). A 1 mm-long incision was made on the cranial side of the flap as a marker and the flap was suspended in cold sterile phosphate-buffered saline (Dulbecco's PBS(-), Nissui Pharmaceutical Co., Ltd.).

After a B6 host mouse was anesthetized with pentobarbital (37.5 mg/kg body weight, i.p.), the right dorsal region was plucked of hairs with fingers and further depilated with said depilatory cream (3.0x3.5 cm) and disinfected with 70% alcohol to prepare an operating field

for skin grafting.

On the denuded area, the BALB/c skin flap prepared above was placed with the marker disposed caudad and sutured in 8 stitches (in the center of each side and at the 4 corners) using a nylon suture with a 6-0 needle (Ethilon; Ethicon Inc.). The surface of the skin graft was covered with a patch of gauze carrying a fradiomycin sulfate ointment (2.0x2.5 cm, Sofratulle; Japan Roussel Co., Ltd.) and further occluded with an adhesive elastic bandage (Elatex; Alcare Co., Ltd.).

The check for engraftment was started at week 2 after transplantation.

(6) Results

The results are shown in Table 1.

Table 1

	Tolerance procedure		Engraftment of skin graft	
	p.v.	i.v	Time after grafting	Engraftment rate (%)
Test group 1	Spleen Cells	Marrow Cells	36	100 (10/10)
Test group 2	Spleen Cells	Spleen Cells	18	20 (1/5)
Test group 3	Marrow Cells	Marrow Cells	36	67 (4/6)
Control group 1	Spleen Cells	-	3	0 (0/4)
Control group 2	Marrow Cells	-	3	0 (0/4)

(7) Explanation of results and discussion

Test group 1: BALB/c mouse-derived spleen cells were administered into the portal vein of 10 MHC-incompatible B6 mice. At day 5 after administration, BALB/c mouse bone marrow cells were injected intravenously, and at day 7, skin grafting was performed. As a result, engraftment of the transferred skin material was confirmed in 10 of 10 mice at week 36 after transplantation.

Test group 2: BALB/c mouse-derived spleen cells were administered into the portal vein of 5 B6 mice and at day 5 after administration, BALB/c mouse-derived spleen cells were injected intravenously. Skin grafting was performed at day 7. As a result, engraftment was confirmed in 1 of 5 mice at week 18 after transplantation but the graft was rejected (dislodged) in one mouse at week 6 and in 3 mice at week 7.

Test group 3: BALB/c mouse bone marrow cells were administered into the portal vein of 6 B6 mice, and at day 5, BALB/c mouse-derived bone marrow cells were administered intravenously. Skin grafting was performed at day 7. As a result, engraftment of the transferred skin was found in 4 of 6 mice at week 36 after transplantation.

Control group: BALB/c mouse-derived spleen cells were administered into the portal vein of 4 B6 mice and skin grafting was performed at day 7. As a result, the graft

was rejected (dislodged) in 2 mice at week 2 after transplantation and in the remaining 2 mice at week 3. Control group 2: BALB/c mouse-derived bone marrow cells were administered into the portal vein of 4 B6 mice and skin grafting was performed at day 7. As a result, the skin graft was rejected in 2 mice at week 2 after transplantation and in the remaining 2 mice at week 3.

It is clear from the above results that the portal administration of the first pharmaceutical composition and subsequent intravenous administration of the second pharmaceutical composition ensure a successful engraftment of the donor's skin graft (maintenance of the donor's alloantigen-specific immunotolerance).

Furthermore, when an immunosuppressant was administered between the portal administration (day 0) and intravenous administration (day 5) of bone marrow cells in the above test group 3, an improvement was obtained in the engraftment rate of transferred skin grafts. The following test examples will cast more light on the above findings.

Test Example 2

(1) Preparation of bone marrow cells and administration into the portal vein and by the intravenous route

Bone marrow cells were prepared and administered in the same manner as the above Test Example 1-(2), (3) and

093057 0907
562050 52508E60

(4).

(2) Administration of an immunosuppressant

As the immunosuppressant, either cyclosporin A (CsA; Sandimmun, 250 mg/5 ml solution, Novartis Pharma K.K.) 10 mg/kg body weight or FK506 (10 mg/ml solution, Fujisawa Pharmaceutical Co., Ltd.) 1 mg/kg body weight was administered intraperitoneally at day 2 and day 5 after portal administration.

(3) Skin grafting

Skin grafting was performed in the same manner as Test Example 1-(5).

(4) Results

The results are shown below in Table 2.

Table 2

	Tolerance procedure			Engraftment of skin graft	
	p.v.	Immuno-suppressant	i.v	Time (W) after grafting	Engraftment rate (%)
Test group 3	Marrow Cells	-	Marrow Cells	36	67 (4/6)
Test group 4	Marrow Cells	CsA	Marrow Cells	32	80 (4/5)
Test group 5	Marrow Cells	FK506	Marrow cells	30	83 (5/6)
Control group 2	Marrow Cells	-		3	0 (0/4)

(5) Explanation of results and discussion

Test group 3 and control group 2 have been fully described in the section of Test Example 1.

Test group 4: BALB/c mouse-derived bone marrow cells were administered into the portal vein of 5 B6 mice. At day 2 and day 5, CsA was administered. In addition, BALB/c mouse-derived bone marrow cells were administered intravenously at day 5 and skin grafting was performed at day 7. As a result, the skin graft was rejected in 1 mouse at week 6 after transplantation but engraftment was obtained in 4 of 5 mice at week 32 after transplantation.

Test group 5: BALB/c mouse-derived bone marrow cells were administered into the portal vein of 6 B6 mice. At day 2 and day 5, FK506 was administered. In addition, BALB/c mouse-derived bone marrow cells were administered intravenously at day 5 and skin grafting was performed at day 7. As a result, the skin graft was rejected in 1 mouse at week 6 after transplantation but engraftment was obtained in 5 of 6 mice at week 30 after transplantation.

The following conclusion can be drawn from the above findings. Although it was generally acknowledged that immunosuppressants such as CsA and FK506 are not suited for use in combination with a tolerogen for the induction of immunotolerance, the use of an immunosuppressant in combination with the portal administration of the first

09380579-090793
662060-62503E60

pharmaceutical composition and the intravenous administration of the second pharmaceutical composition in accordance with this invention results in an improved engraftment rate and is, therefore, effective in inducing immunological tolerance.

Thus, in accordance with this invention, there is provided a new immunotolerance inducing technique which can be fully expected to find clinical application.

Test Example 3

(1) Preparation of bone marrow cells and the portal and intravenous injection of the cells

The procedures described in Example 1-(2), (3) and (4) were repeated.

(2) Administration of an immunosuppressant

CsA, 10 mg/kg body weight, was administered intraperitoneally at day 2 and day 5 after portal injection.

(3) Skin grafting

Except that skin grafting was performed on the same day as the portal administration, the procedure of Test Example 1-(5) was repeated (n=6). A control group receiving skin flaps derived from C3H mice was also provided (n=4).

(4) Results

The results are shown in Fig. 1.

66/060" 5/503E50

In Fig. 1, the ordinate represents the engraftment rate (%) of skin flaps and the abscissa represents time (in weeks) after grafting. Group 1 is a test group and Group 2 is a control group.

The results of this test example indicate that the immunotolerance inducing procedure comprising administration of the first pharmaceutical composition into the portal vein and the organ transplantation can be concurrently carried out. Therefore, in humans, too, the portal administration of the first pharmaceutical composition (bone marrow and other cells) from the donor and the organ transplantation can be concurrently performed. This technique is considered to be an epochal one in that the graft vs. host reaction (GvH reaction) can be prevented even without removal of T cells from the marrow cell fraction and the immunotolerance can be sufficiently maintained using only two doses of an immunosuppressant.

Test Example 4

The induction of immunotolerance was carried out by the portal or intravenous injection of an allogeneic donor's bone marrow cells and the establishment of immunological tolerance was evaluated using the engraftment rate of the skin grafts (allogeneic to the donor) which are most susceptible to rejection as an indicator.

(1) Preparation of a bone marrow cell suspension

From the donor mouse, the femurs and tibias were removed and a 22 G needle (Code No. NN-2225R, Terumo Co., Ltd.) attached to a syringe (2.5 ml, Code No. SS-02S, Terumo Co., Ltd.) was inserted into each bone from the knee-joint side. The bone marrow cells were flushed into a sterilized dish (90x15 mm, Iwaki Clinical Test Ware) using RPMI1640 solution from the syringe and suspended in RPMI1640 solution. The harvested bone marrow cells were washed with RPMI1640 solution once and resuspended in the same solution to provide the objective bone marrow cell suspension (concentration: 1×10^8 cells/ml).

(2) Radiation

Irradiation of the recipient mice was carried out by the TBI method using Gamma Cell 40 Exacter (Nordion International Inc.) and ^{137}Cs as a beam source.

(3) Portal administration

The recipient mouse was shaved of hairs with a razor under pentobarbital anesthesia (Pitman-Moor Inc.; 37.5 mg/kg body weight, i.p.) and after disinfection, a midline incision was made in the abdomen and the mesenterium was exposed. A 27 G needle (Terumo Co., Ltd.) attached to a 1 ml-tuberculin syringe was inserted through the adipose tissue of the mesenterium and 3×10^7 bone marrow cells from the donor mouse (0.3 ml of the suspension prepared above)

were administered into the portal vein.

(4) Intravenous administration

The bone marrow cell suspension prepared above from the donor mouse was adjusted to 1×10^8 cells/ml and the 3×10^7 equivalent thereof (0.3 ml) was administered from the tail vein of the recipient mouse.

(5) Skin grafting

The preparation and transplantation of skin grafts were carried out as follows, with reference to the procedures described in the literature [Mayumi et al., Jpn. J. Surg., 18, 548-557 (1988)].

Thus, the donor mouse was sacrificed under ethyl ether (Nacalai Tesque Inc.) anesthesia and the whole hair coat was removed with a depilatory cream (Feather Hair Remover, Feather Safety Razor Co. Ltd.). After disinfection with 70% alcohol solution, the full-thickness skin layer was peeled off and recovered. Using a pair of forceps (with a bent tip, tapered, non-toothed) and sanitary cotton balls, the subcutaneous fat tissue was removed as much as possible and the skin was cut into a flap (1.2x1.5 cm square). A 1 mm-incision was made on the cranial side of the flap as a marker and the skin flap was left floating in cold sterilized phosphate-buffered saline (Dulbecco's PBS(-), Nissui Pharmaceutical Co. Ltd.).

After the recipient mouse was anesthetized with pentobarbital (37.5 mg/kg body weight, i.p.), the right dorsal region was plucked of hairs with fingers and further depilated with said depilatory cream (3.0x3.5 cm). The denuded area was disinfected with 70% alcohol solution to prepare an operating field for skin grafting.

On the denuded area, the donor's skin flap prepared above was placed with the marker disposed caudad and sutured in 8 stitches (in the center of each side and at the 4 corners) using a nylon suture with a 6-0 needle (Ethilon; Ethicon Inc.). The surface of the graft was covered with a patch of gauze carrying a fradiomycin ointment (2.0x2.5 cm, Sofratulle; Japan Roussel Co., Ltd.) and further occluded with an adhesive elastic bandage (Elatex; Alcare Co., Ltd.).

(6) Induction of immunological tolerance

Using (BALB/cxDBA2) F1 mice (H-2K^d) (aged 7~8 weeks, 19~20 g, Japan SLC) as donor mice and B6 mice (H-2K^b) (aged 10~13 weeks, 20~23 g, Japan SLC) as recipient mice, each recipient animal was irradiated and, after 1 day, the donor's bone marrow cells were administered either into the portal vein or intravenously. Skin grafting was performed within the same day as the portal or intravenous administration of bone marrow cells and the check for engraftment of skin flaps was made starting week 3 after

052050" 62503E60

transplantation.

(7) Results

The results are shown in Fig. 2.

In Fig. 2, the ordinate represents engraftment rate (%) and the abscissa represents time (in weeks) after transplantation. The legend Group I represents the data generated in a group (n=3) which received a radiation dose of 6.5 Gy in association with portal administration of bone marrow cells [Group I: 6.5 Gy+pv (n=3)]; the legend Group II represents a group which received a radiation dose of 7.0 Gy in association with portal administration (n=9) or intravenous administration (n=5) of bone marrow cells [Group II: 7 Gy+pv (n=9) or iv (n=5)]; the legend Group III represents a group which received a radiation dose of 6.5 Gy in association with intravenous administration of bone marrow cells (n=7) [Group III: 6.5 Gy+iv (n=7)]; and the legend Group IV represents a group which received a radiation dose of 6.0 Gy in association with portal administration (n=5) or intravenous administration (n=3) of bone marrow cells [Group IV: 6.0 Gy+pv (n=5) or iv (n=3)].

(8) Explanation of the results

In B6 mice, total body irradiation was performed in a dose of 7.0 Gy, 6.5 Gy or 6.0 Gy and after about 24 hours, the portal (pv) or intravenous (iv) injection of bone

662060" 64503E60

marrow cells derived from a (BALB/cxDBA/2) F1 mouse (CDF1) was carried out. Then, within the same day, skin grafting was performed. As shown in Fig. 2, the recipient mice given a radiation dose of 7 Gy in both the portal and intravenous administration groups showed an engraftment rate of 100% for the donor (CDF1)'s skin graft at week 23 (on the 167th day) after transplantation (9 of 9 mice in the pv group and 5 of 5 mice in the iv group). This is in contrast with the recipient mice exposed to a radiation dose of 6.0 Gy, in which the skin graft was invariably rejected within 3 weeks after transplantation (5 of 5 mice in the pv group and 3 of 3 mice in the iv group). In the recipient mice given a radiation dose of 6.5 Gy, the skin graft was rejected in one of 7 mice in the intravenous administration group at week 3 after transplantation but successful engraftment was obtained in 3 of the 3 recipient mice in the portal administration group at week 13 after transplantation.

(9) Discussion

The engraftment rate was slightly higher in the 6.5 Gy plus portal administration group. It appears that because the donor's hematopoietic stem cells are trapped in the recipient's liver with higher efficiency in this group, the rejection by radio-resistant immunocompetent cells in the recipient mice is more effectively avoided.

Pharmaceutical Example 1

Bone marrow cells or spleen cells are suspended in physiological saline to prepare a 1×10^8 /ml composition for administration into the portal vein. On the other hand, a composition containing 1×10^8 bone marrow cells/ml saline is similarly prepared for intravenous administration.

In humans, the above composition for portal administration is preferably administered in a dose of generally 3×10^8 bone marrow cells or more (T cells may be present) per kg body weight.

Pharmaceutical Example 2

Bone marrow cells are suspended in physiological saline to provide a 1×10^8 /ml suspension. For administration into the portal vein of a patient, for instance, the suspension is preferably administered in a dose of generally 3×10^8 bone marrow cells or more (a small proportion, i.e. about 2%, of T cells may be present) per kg body weight. Thus, there is provided an injection containing at least the above unit dose. This injectable composition is of value as an immunotolerance inducer to be used in association with radiation.

INDUSTRIAL APPLICABILITY

With the immunotolerance inducer of this invention, an immunological tolerance can be induced in a patient undergoing an organ transplantation and a positive

09380579 1050759

maintenance of the transplanted organ can therefore be ensured.

65/060" 6/503E.60

CLAIMS

1. An immunotolerance inducer for inducing immunological tolerance in a patient undergoing an organ transplantation which comprises a first pharmaceutical composition for portal administration which comprises an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells, mature lymphocytes or a mixture thereof in combination with a pharmaceutical carrier and a second pharmaceutical composition for intravenous administration which comprises an effective amount of said tolerogen in combination with a pharmaceutical carrier.

2. The immunotolerance inducer according to Claim 1 wherein the tolerogen is comprised of bone marrow cells.

3. An immunotolerance inducer for inducing immunological tolerance in a patient undergoing an organ transplantation which is to be used in association with radiation, comprising an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells or a mixture thereof in combination with a pharmaceutical carrier.

4. The immunotolerance inducer according to Claim 3 wherein the tolerogen is comprised of bone marrow cells.

5. A method of inducing immunological tolerance in

552060 52503E60

0030059 090930
664060 62500560

a patient undergoing an organ transplantation in whom an induction of immunological tolerance is needed, which comprises a step of portal administration of a first pharmaceutical composition for portal administration comprising an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells, mature lymphocytes or a mixture thereof in combination with a pharmaceutical carrier and a step of intravenous administration of a second pharmaceutical composition for intravenous administration comprising an effective amount of said tolerogen in combination with a pharmaceutical carrier.

6. The method according to Claim 5 further comprising administering an immunosuppressant drug during the interim period between the portal administration and intravenous administration.

7. A method of inducing immunological tolerance in a patient undergoing an organ transplantation in whom an induction of immunological tolerance is needed, which comprises subjecting the patient to radiation and administering a pharmaceutical composition comprising an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells or a mixture thereof in combination with a pharmaceutical carrier.

8. Use of a tolerogen containing hematopoietic stem

cells, hematopoietic progenitor cells, mature lymphocytes or a mixture thereof in the production of the immunotolerance inducer of Claim 1 or 3 for the induction of immunological tolerance in a patient undergoing an organ transplantation.

66/060" 6/20/86

ABSTRACT

652060" 62503E60

This invention provides a novel immunotolerance inducer which is effective in inducing immunological tolerance without involving any remarkable invasive procedure, thus ensuring the maintenance of a transplanted organ without administration of an immunosuppressant drug. This immunotolerance inducer is either a dual-system inducer comprising a first pharmaceutical composition for portal administration which comprises an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells, mature lymphocytes or a mixture thereof as the active component and a second pharmaceutical composition for intravenous administration which comprises an effective amount of the above-mentioned tolerogen as the active component or a single-system immunotolerance inducer which comprises an effective amount of a tolerogen containing hematopoietic stem cells, hematopoietic progenitor cells or a mixture thereof as an active component, said single-system immunotolerance inducer being administered in association with radiation.

FIG. 1

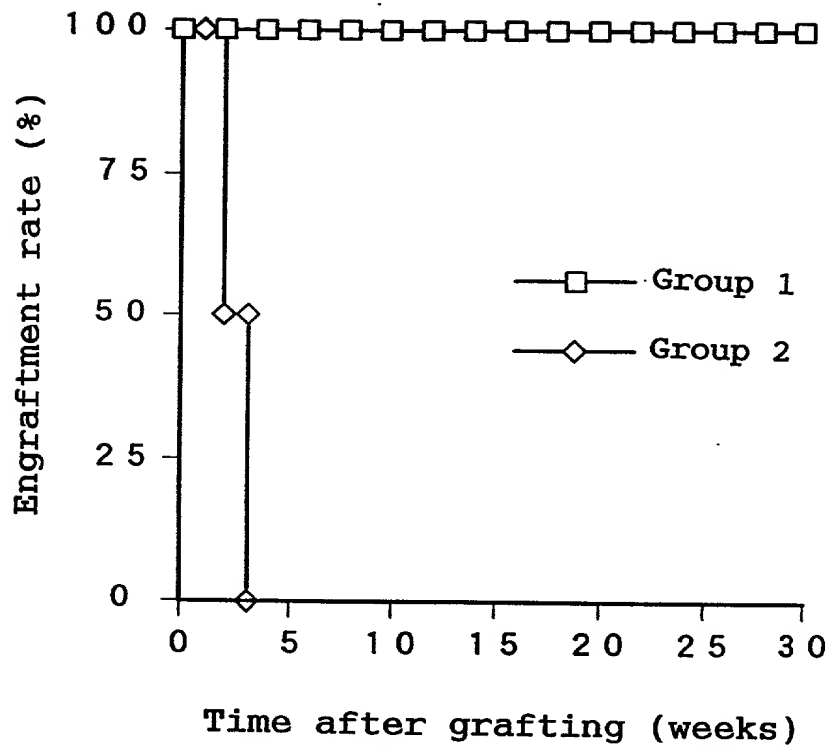
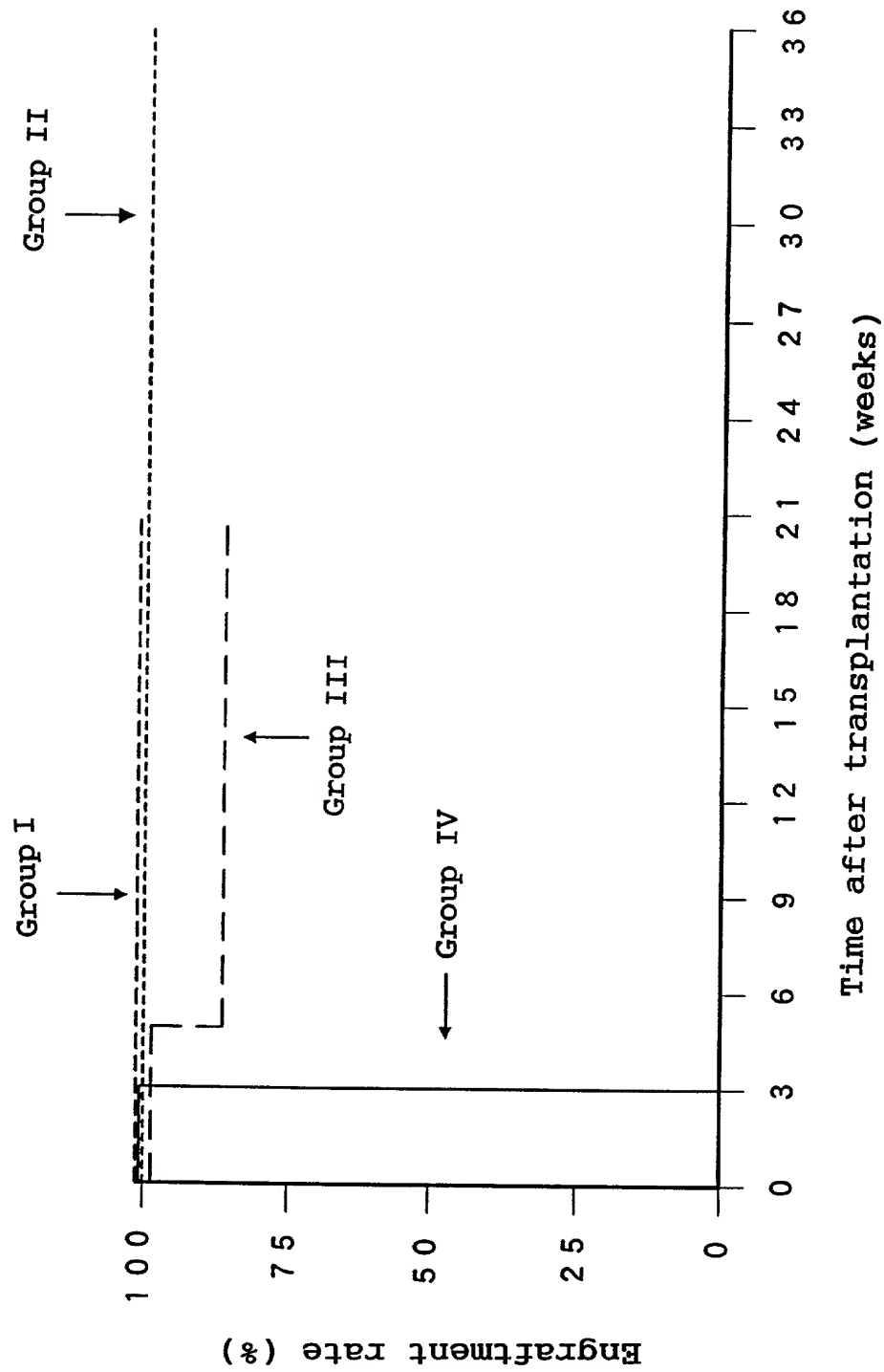


FIG. 2



Japanese Language Declaration

(日本語宣言書)

私は、米国法典第35編第119条(a)-(d)項又は第365条(b)項に基づき下記の、米国以外の国の少なくとも一カ国を指定している特許協力条約第365条(a)項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

Prior Foreign Applications

外国での先行出願

52930/1997	Japan
(Number)	(Country)
(番号)	(国名)
155015/1997	Japan
(Number)	(Country)
(番号)	(国名)
346737/1997	Japan
(Number)	(Country)
(番号)	(国名)

私は、第35編米国法典119条(e)項に基づいて下記の米国特許出願規定に記載された権利をここに主張致します。

(Application No.)	(Filing Date)
(出願番号)	(出願日)

私は、下記の米国法典第35編第120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約第365条(c)に基づく権利をここに主張します。又、本出願の各請求範囲の内容が米国法典第35編第112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内又は特許協力条約国際出願提出日までの期間中に入手された、連邦規則法典第37編第1条第56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

(Application No.)	(Filing Date)
(出願番号)	(出願日)

(Application No.)	(Filing Date)
(出願番号)	(出願日)

私は、私自身の知識に基づいて本宣言中で私が行う表明が真実であり、かつ私の入手した情報と私の信ずるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行えば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby claim foreign priority under Title 35, United States Code, Section 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Priority Not Claimed

優先権主張なし

07/03/1997	<input type="checkbox"/>
(Day/Month/Year Filed)	
(出願年月日)	
12/06/1997	<input type="checkbox"/>
(Day/Month/Year Filed)	
(出願年月日)	
16/12/1997	<input type="checkbox"/>
(Day/Month/Year Filed)	
(出願年月日)	

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.)	(Filing Date)
(出願番号)	(出願日)

I hereby claim the benefit of Title 35, United States Code Section 120 of any United States application(s), or 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose any material information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Status: Patented, Pending, Abandoned)
(現況: 特許許可済、係属中、放棄済)

(Status: Patented, Pending, Abandoned)
(現況: 特許許可済、係属中、放棄済)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declaration and Power of Attorney for Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name,

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

IMMUNOTOLERANCE INDUCER

上記発明の明細書（下記の欄でX印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ ____月 ____日に提出され、米国出願番号または特許協定条約

☒ was filed on March 4, 1998
as United States Application Number or
PCT International Application Number

国際出願番号を _____ とし、

PCT/JP98/00909 and was amended on

（該当する場合） _____ に訂正されました。

_____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration

(日本語宣言書)

委任状: 私は、下記の発明者として、本出願に関する一切の
手続きを米国特許商標局に対して遂行する弁理士又は代理
人として、下記のことを指名致します。(弁護士、又は代理人の
氏名及び登録番号を明記のこと)

POWER OF ATTORNEY: As a named inventor, I hereby
appoint the following attorney(s) and/or agent(s) to
prosecute this application and transact all business in the
Patent and Trademark Office connected therewith (list
name and registration number)

John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092;
Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank
Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B.
Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch,
Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L.
Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon
Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland,
Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon,
Reg. No. 32,778; Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg.
No. 33,102 and Brett S. Sylvester, Reg. No. 32,765

29

書類送付先:

Send Correspondence to:

-SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037-3202

直通電話連絡先: (名称及び電話番号)

Direct Telephone Calls to: (name and telephone number)

(202)293-7060

唯一又は第一発明者名	Full name of sole or first inventor
発明者の署名	Inventor's signature
住所	Residence
国籍	Citizenship
郵便の宛先	Post office address
第二共同発明者名 (該当する場合)	Full name of second joint inventor, if any
第二発明者の署名	Second inventor's signature
住所	Residence
国籍	Citizenship
郵便の宛先	Post office address

(第三以降の共同発明者についても同様に記載し、署名をする (Supply similar information and signature for third and subsequent joint inventors.))

Japanese Language Declaration
(日本語宣言書)

第三共同発明者名 (該当する場合)		Full name of third joint inventor, if any	
第三発明者の署名	日付	JIN Tienan	Date
	300	Tienan Jin	August 25, 1999
住所		Residence Osaka-shi, Osaka 536-0012, Japan	JPX
国籍		Citizenship China	
郵便の宛先		Post office address 29-401, Tennoden 4-chome, Joto-ku, Osaka-shi, Osaka 536-0012, Japan	
第四共同発明者名 (該当する場合)		Full name of fourth joint inventor, if any	
第四発明者の署名	日付	MORITA Haruo	Date
	2/00	Haruo Morita	August 25, 1999
住所		Residence Osaka-shi, Osaka 535-0003, Japan	JPX
国籍		Citizenship Japan	
郵便の宛先		Post office address 2-14-5, Nakamiya, Asahi-ku, Osaka-shi, Osaka 535-0003, Japan	
第五共同発明者名 (該当する場合)		Full name of fifth joint inventor, if any	
第五発明者の署名	日付	SOGO Shinji	Date
	500	Shinji Sogo	August 25, 1999
住所		Residence Tokushima-shi, Tokushima 770-0943, Japan	JPX
国籍		Citizenship Japan	
郵便の宛先		Post office address 2-39-1-205, Nakashowa-cho, Tokushima-shi, Tokushima 770-0943, Japan	
第六共同発明者名 (該当する場合)		Full name of sixth joint inventor, if any	
第六発明者の署名	日付	YAMANISHI Kazuya	Date
	600	Kazuya Yamanishi	August 25, 1999
住所		Residence Tokushima-shi, Tokushima 770-8012, Japan	JPX
国籍		Citizenship Japan	
郵便の宛先		Post office address 19-139, Higashichiyogamaru, Obara-cho, Tokushima-shi, Tokushima 770-8012, Japan	

Japanese Language Declaration
(日本語宣言書)

第七共同発明者名 (該当する場合)		Full name of seventh joint inventor, if any	
第七発明者の署名	日付	ADACHI Masakazu	
住所		Seventh inventor's signature	Date
国籍		August 25, 1999	
郵便の宛先		Residence	
		Takasaki-shi, Gunma 370-0864, Japan	
		Citizenship	
		Japan	
		Post office address	
		3493-9, Ishihara-machi, Takasaki-shi, Gunma 370-0864, Japan	
第八共同発明者名 (該当する場合)		Full name of eighth joint inventor, if any	
第八発明者の署名	日付	Eighth inventor's signature	
住所		Date	
国籍		Residence	
郵便の宛先		Citizenship	
		Post office address	
第九共同発明者名 (該当する場合)		Full name of ninth inventor, if any	
第九発明者の署名	日付	Ninth inventor's signature	
住所		Date	
国籍		Residence	
郵便の宛先		Citizenship	
		Post office address	
第十共同発明者名 (該当する場合)		Full name of tenth joint inventor, if any	
第十発明者の署名	日付	Tenth inventor's signature	
住所		Date	
国籍		Residence	
郵便の宛先		Citizenship	
		Post office address	